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***FLNA* variants associated with disorders of platelet number or function**

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Gene and protein structure and function

FLNA is a 48-exon gene located at chromosome Xq28 that encodes the 280 kDa cytoskeletal protein filamin A (FLNa; UniProt P21333)^{1,2}. The mature FLNa protein of 2646 amino acids comprises an amino terminal actin-binding domain (ABD) and 24 immunoglobulin-like domains (IGDs).^{3,4} The IGDs contribute to rod region 1 (IGDs 1-15) which facilitates actin binding and rod region 2 (IGDs 16-23) which interacts with multiple binding partners. IGD 24 at the carboxyl terminus mediates assembly of FLNa monomers into functional homodimers.³

The role of FLNa in megakaryocytes and platelets

FLNa is widely expressed and interacts with over 90 other proteins, notably components of the cytoskeleton, transmembrane receptors and downstream signaling mediators (specifically reviewed in platelets in ⁵). For example, inhibitory interactions with FLNa maintain integrin $\alpha_{IIb}\beta_3$ in an inactive state, thereby regulating normal proplatelet formation from megakaryocytes (MK) and the dynamics of activation of mature platelets.⁶ The FLNa interaction with PACSIN2 regulates membrane tubulation in MK and platelets through appropriate localization of PACSIN2 to internal membrane systems.⁷ In platelets, the FLNa-GPIIb/IIIa interaction regulates downstream signaling following von Willebrand factor binding and contributes to membrane integrity and platelet adhesion in high shear conditions.^{8,9} FLNa interactions also support platelet activation responses by enabling the spatial localization of Syk to the cytoplasmic membrane and ITAM / ITAM-like containing receptors such as GPVI.¹⁰

The filaminopathies A

Pathogenic variants in *FLNA* are implicated in several rare human disorders collectively referred to as the filaminopathies A.¹¹ The best described of these is periventricular nodular heterotopia 1 (PVNH1:OMIM #300049) in which defective neuronal migration results in formation of nodular tissue surrounding the cerebral ventricles, usually manifesting as epilepsy, sometimes also associated with cardiac, connective tissue, gastrointestinal, lung and other abnormalities.¹² PVNH1 is an X-linked dominant disorder in which almost all reported cases are heterozygous females. Pre or peri-natal death is common in hemizygous males. Surviving males have a severe multisystem developmental disorder or are somatic mosaics for pathogenic *FLNA* variants.^{12,13} *FLNA* variants that cause PVNH1 are typically high-impact and confer loss of FLNa function, or copy number variants, but some are missense loss-of-function variants, usually affecting the ABD.¹⁴ The filaminopathy A termed chronic idiopathic intestinal pseudo-obstruction (CIIP:OMIM #300048) is also associated with loss-of-function *FLNA* variants and may be an alternatively expressed form of PVNH1.¹⁵

Prevalence of platelet abnormalities in the filaminopathies A

Amongst the 170 evaluated reports of PVNH1 or CIIP associated with pathogenic *FLNA* variants, thrombocytopenia with or without platelet dysfunction is documented in 11 cases from 10 pedigrees with a total of 10 different *FLNA* variants. These include four stop-gain variants, one insertion deletion variant and five copy number variants that were incompletely characterized (Figure 1)¹⁴⁻²². There are five reported cases in which a normal platelet count is documented^{13,16-18,23}. For the remainder of cases, no hematological data are reported. There is a single reported case with a missense *FLNA* variant and thrombocytopenia but without other features of PVNH1,

although the pathogenicity of this variant has not been confirmed experimentally.^{16,24} Together these observations suggest that thrombocytopenia is an incompletely expressed phenotype of PVNH1 and CIIP. Other rare filaminopathies A caused by gain-of-function *FLNA* variants (Table 1) are not associated with thrombocytopenia.

Platelet abnormalities associated with *FLNA* variants

In the PVNH1 or CIIP cases with thrombocytopenia, reported symptoms include menorrhagia, mucocutaneous hemorrhage, slow wound healing and prolonged bleeding times^{16,18,19,21}. In affected cases, the median reported platelet count was 80 (32-140 x10⁹/L). Thrombocytopenia was associated with enlarged, spherical platelets^{16,17} with abnormal α -granule distribution compared with controls.¹⁶ Platelets also showed abnormal cytoplasmic distribution of FLNa and reduced total FLNa content consistent with diminished total expression of FLNa.¹⁶ Other reports have demonstrated FLNa expression is absent in a platelet sub-population, likely reflecting selective expression of the variant allele only in specific platelets.^{18,24} MKs from patients with loss-of-function *FLNA* variants have shown defective differentiation and structural fragility, with abnormal distribution of FLNa in the cytoplasm^{16,24}. **MKs generated *ex vivo* from pluripotent stem cells from filaminopathy A cases showed incomplete MK maturation and the production of reduced numbers and enlarged platelets and associated with dysregulated RhoA activation.**⁶

In three cases with loss-of-function *FLNA* variants and a PVNH1-thrombocytopenia phenotype, platelets showed reduced aggregation, secretion and glycoprotein VI signaling responses to type 1 collagen and to convulxin, and also diminished thrombus formation on collagen.^{16,24} Responses to other activation agonists were

similar to controls suggesting a selective defect in GPVI signal transduction, consistent with diminished FLNa-Syk localisation to the GPVI complex.²⁴ A patient with confirmed Bernard Soulier syndrome harbouring an additional *FLNA* variant (p.V528M) was also found to have abnormal platelet functional responses to collagen. Although the authors postulated the *FLNA* variant directly contributed to the abnormal platelet function phenotype²⁵, the pathogenicity of the p.V528M alone is questionable because this variant is present in approximately one in 340 alleles in unselected populations.²⁶ In another case with features of PVNH1 and CIIP associated with an *FLNA* variant predicting a C-terminal extension of FLNa, platelets were normal in number but demonstrated increased aggregation, secretion and thrombus formation compared to controls when stimulated with a variety of agonists. These changes were attributed to altered interactions between variant FLNa and the $\alpha_{IIb}\beta_3$ integrin.²³

Implications and conclusions

The heritability of filaminopathy A-associated thrombocytopenia has important implications for heterozygous female carriers of *FLNA* variants, because of the risk of a lethal or otherwise severe phenotype in hemizygous male offspring. Appropriate preconceptual counselling is essential for women with genotypically proven filaminopathy A. However, the possibility of filaminopathy A should also be considered in women in which thrombocytopenia may be the only apparent feature of filaminopathy A unless central nervous system imaging or other specialist investigations are performed. In this circumstance, comprehensive genotyping and accurate assessment of the pathogenicity of any observed *FLNA* variants is essential for appropriate counselling of disease risk. Although pathogenicity can

confidently be assigned to high impact *FLNA* variants, currently there is insufficient evidence to confidently predict the effects of missense variants **without further systematic reporting of genotype-phenotype associations with supporting functional evaluation.**

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Contributions

All authors reviewed the literature, co-wrote the manuscript and assembled the figure.

Disclosures

The authors have no conflicts of interest to disclose.

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nodular heterotopia nor with macrothrombocytopenia. Journal of Human Genetics. Vol 55: Nature Publishing Group; 2010:844-6.

Table 1.

Variant effect	Disorder [OMIM ID]	Clinical features	Thrombocytopenia reported
Loss of function	X-linked dominant periventricular nodular heterotopia [PVNH1 #300049]	Abnormal neuronal migration, periventricular nodules, seizures, congenital cardiac defects	Yes
	Chronic idiopathic intestinal pseudo-obstruction, congenital short bowel syndrome [CIIP #300048]	Recurrent symptoms and signs of bowel obstruction in the absence of mechanical lesions, short bowel	Yes
Gain of function	Otopalatodigital syndrome 1 [#311300]	Cleft palate, skeletal abnormalities, deafness	No
	Otopalatodigital syndrome 2 [#304120]	Skeletal abnormalities, organ malformation, frequent prenatal death	No
	Frontometaphyseal dysplasia [#305620]	Skeletal dysplasia, deafness, urogenital defects	No
	Melnick-Needles syndrome [#309350]	Severe skeletal dysplasia, prenatal and early death in males	No

Table 1: Disorders associated with pathogenic variants in *FLNA* (filaminopathies A), divided by reported effects of variant on protein function. Ultra-rare disorders in which *FLNA* variants have been implicated in only a minority of cases are not listed here.

Figure 1 Legend

- A. Schematic representation of filamin A (UNIPROT 21333) monomer showing the actin binding (ABD), calponin-homology (CH1 and CH2) and immunoglobulin-like (1-24) domains and hinge regions 1 and 2 (H1 and H2). The amino acid residues delimiting the domains are indicated. The arrows below the schematic show the amino acid location of stop gain and frameshift *FLNA* variants (incompletely characterised variants not shown).
- B. Previously reported *FLNA* variants associated with thrombocytopenia. Where more than one family member is affected, data is presented for the index case. Population estimates allelic frequencies for the missense variants were obtained from the GnomAD database <https://gnomad.broadinstitute.org/>. Age at time of report is stated. NR- clinical or platelet phenotype not reported; PVNH1- periventricular nodular heterotopia; CIIP- chronic idiopathic intestinal pseudo-obstruction. UA- unable to ascertain. *The pathogenicity of this variant was not confirmed by functional analyses. # “Thrombocytopenia” reported but no platelet count stated.